NOTES

EVALUATION OF A FILTER BAG SYSTEM FOR NDF, ADF, AND IVDMD FORAGE ANALYSIS

KENNETH P. VOGEL,* JEFFREY F. PEDERSEN, STEVEN D. MASTERSON, AND JOHN J. TOY

Abstract

A new method of determining in vitro dry matter digestibility (IVDMD) was recently developed in which the digestion is conducted with the forage samples in filter bags. Our objective was to compare the filter bag and conventional IVDMD analysis methods using smooth bromegrass (*Bromus inermis* Leyss.), switchgrass (*Panicum virgatum* L.), and forage sorghum [*Sorghum bicolor* (L.) Moench] samples. In addition, the filter bag analysis systems for determining non-sequential neutral and acid detergent fiber (NDF and ADF), respectively, were compared with the non-sequential conventional analysis systems. In the filter bag systems, the forage samples are sealed in filter bags and the analyses are conducted on a batch basis rather than on an individual basis as in the conventional IVDMD and fiber analysis procedures. The filter bag analysis methods produced results similar to the conventional methods and ranked the forage samples in the same relative order.

A FILTER BAG METHOD of analyzing forages for NDF, ADF, lignin, and in vitro true digestibility was developed recently by ANKOM Technology Corporation[1] (Fairport, NY). In the ANKOM system, forage samples in individual filter bags are processed in bulk containers rather than in individual sample digestion tubes or filtration units. In the ANKOM in vitro true digestibility procedure, a rumen fluid digestion is followed by a neutral detergent fiber (NDF) digestion (Anonymous, 1995b, Traxler et al., 1995) which differs from the conventional in vitro dry matter digestibility (IVDMD) procedure. In the conventional IVDMD procedure, the rumen fluid digestion period is followed by a 48-h acid pepsin digestion (Marten and Barnes, 1980). Forage breeders have used the IVDMD procedure successfully to develop forage cultivars with improved digestibility (Vogel and Sleper, 1994). Because of this success, forage breeders will likely continue to use IVDMD procedure in their breeding programs. Procedures for using the ANKOM filter bag system for IVDMD analysis and their validation have not been published to date. Only limited information is available that compares ANKOM system and conventional NDF and ADF results and is primarily from ANKOM Corporation or in brief abstracts (Komarek, 1993; Komarek et al., 1994)

A principal objective of this study was to evaluate a method we developed for determining in vitro dry matter digestibility (IVDMD) using filter bags in the ANKOM Rumen Fermenter by comparing the digestion results with those from the conventional IVDMD procedure. In addition, the same set of forage samples were used to provide an independent assessment of the validity of filter bag analysis systems for determining neutral and acid detergent fiber (NDF and ADF) by comparing results from filter bag and conventional NDF and ADF analysis.

Materials and Methods

The forage samples used in this study (Table 1) included first and second harvest or cut (C1 or C2, respectively) samples of four smooth bromegrass cultivars from a variety trial, seven switchgrass samples from a management study in which 'Cave-in-rock' switchgrass plots were harvested at weekly intervals the summer of 1994 starting at the boot stage (H1) to fully senescent (H8) and subsequent regrowth (C2) harvested after a killing frost, and eight forage sorghums harvested at the maturity stage recommended for silage. All forage samples were grown in experiments located at Mead, NE. The samples represent C_3 cool-season perennials, C_4 warm-season perennials, and C_4 warm-season annuals.

All field samples were oven dried at 50°C, ground sequentially in Wiley and UDY mills with 2- and 1-mm screens, respectively, and stored in sealed plastic sample vials. Initial sample dry weight for the ADF and NDF analyses was 0.5 g. For the ADF and NDF conventional analysis, the 0.5-g samples were dried for 22 h at 100°C before weighing to obtain pre-extraction dry weights. The same drying procedure was used to obtain post-extraction dry weights. For the filter bag system, 0.5 g samples were weighed into individual preweighed and numbered filter bags which were then heat sealed. The drying and weighing procedures were subsequently the same as for the conventional analysis. For the conventional and filter bag IVDMD analyses, initial sample dry weights were 0.25 g and 0.50 g, respectively. The weighing procedure for IVDMD procedures was the same as for the NDF and ADF analysis except initial sample weights were determined after drying at 60°C for 48 h and final undigested residue weights were determined after drying the samples 72 h at 60°C.

For the conventional (C) ADF and NDF analysis (ADF-C and NDF-C, respectively), procedures of Goering and Van Soest (1970) were used except that decaline was not used in the ADF and NDF analysis and sodium sufite was not used in the NDF analysis as per recommendations of Van Soest and Robertson (1980). Standard coarse fritted disk gooch crucibles were used for the filtration process. The ANKOM Fiber Ana-

[1] Names of products are included for the benefit of the reader and do not imply the endorsement by USDA or the Univ. of Nebraska.

USDA-ARS, 344 Keim Hall, Dep. of Agronomy, Univ. of Nebraska, P.O. Box 830937, Lincoln, NE 68583-0937 and Center for Grassland Studies, Univ. of Nebraska, Lincoln, NE. J. series no. 11854. Nebraska Agric. Exp. Stn. Received 11 Nov. 1997. *Corresponding author (agro012@unlvm.unl.edu).

Published in Crop Sci. 39:276–279 (1999).

Abbreviations: NDF, neutral detergent fiber; ADF, acid detergent fiber; IVDMD, in vitro dry matter digestibility or disappearance; NDF-A, NDF with alpha-amylase added to the rinse solution.

Table 1. Means by entry comparing filter bag (FB) vs. conventional (C) forage fiber and digestibility analysis for three classes of forages.

Species	Cultivar or line†	NDF-A‡ FB	NDF FB	NDF C	ADF FB	ADF C	IVDMD FB	IVDMD C
		g kg^{-1}						
Bromegrass	Fleet C1	563	654	620	328	382	674	636
	Lincoln C1	587	655	656	351	355	647	632
	Radison C1	620	660	697	363	383	587	513
	Saratoga C1	563	621	643	336	343	689	656
	Fleet C2	580	675	665	380	412	600	578
	Lincoln C2	575	644	658	364	398	578	506
	Radison C2	587	647	652	375	420	562	514
	Sratoga C2	537	617	642	359	405	611	535
Switchgrass	H1, C1	698	720	764	400	408	618	591
	H2, C1	678	705	763	402	423	565	554
	H4, C1	673	733	758	415	422	521	499
	H8, C1	680	743	768	447	488	382	435
	H1, C2	626	652	704	369	392	517	458
	H2, C2	649	684	721	390	423	521	441
	H4, C2	582	622	659	337	356	633	550
Sorghum forage	Redlan bmr-leaves	635	703	705	415	438	603	551
	Redlan bmr-stems	621	664	677	406	417	610	594
	Redlan leaves	640	729	729	445	490	533	479
	Redlan stems	608	662	648	413	428	577	576
	Greenterat II	643	693	681	389	394	676	645
	Waconia-L	408	509	497	284	300	769	719
	Atlas (without grain)	688	676	708	461	469	694	665
	Atlas (with grain)	489	567	562	336	382	684	628
	Mean	606	662	677	381	406	602	563
	Mean SD§	14	18	16	32	58	18	25

† C1 = cut 1, C2 = cut 2, H1–H8 weekly harvest interval samples with H1 = boot stage and H8 fully senescent, bmr = brown midrib.
‡ NDF-A = neutral detergent fiber with alpha-amylase rinse, NDF = neutral detergent fiber, ADF = acid detergent fiber, IVDMD = in vitro dry matter digestibility.
§ Mean SD: Each mean in table has an associated SD; Mean SD is the mean of these associated standard deviations.

lyzer (Model No: ANKOM 200, Ankom Technology, Fairport, NY) was used for the filter bag (FB) NDF and ADF (NDF-FB and ADF-FB, respectively) analysis (Anonymous, 1995a). The same detergent solutions were used in both the conventional and filter bag fiber analysis procedures. Both conventional and filter bag NDF and ADF analysis were non-sequential. The only difference between the analysis methods was that in the filter bag system, the samples were in filter bags and were bulk processed in a sealed, stainless steel reaction vessel in which the NDF or ADF solution was maintained at a constant temperature with agitation. Filter bags were suspended in the reaction vessel in a stainless steel or plastic suspension basket. ANKOM F57 filter bags were used for ADF and NDF analysis in this study. The 2.5-L reaction vessel holds 2.1 L of ADF or NDF solution that was maintained under slight positive pressure at 99°C. In the filter bag system, the samples were processed in the reaction vessel for 70 min for the ADF procedure and 80 min for NDF.

After the extraction period, the ADF or NDF solution was drained from the reaction vessel, and the reaction vessel was filled with 2 L of 94°C tap water. The top was left open and the samples were agitated for 5 min. The hot water rinse was repeated four times. After the final rinse, the bags were removed and gently squeezed to press out excess water. The bags were then placed in a small beaker and covered with acetone. After 5 min of soaking, the bags were again squeezed to remove the acetone, air dried in a hood, and then dried at 100°C for the final drying step.

ANKOM Technology recommends adding α-amylase to the NDF rinse for samples containing grain including corn (*Zea mays* L.) and sorghum cut for silage such as the 'Atlas' sorghum sample in this study. An alternative filter bag NDF with α-amylase (NDF-A) was evaluated and compared with conventional NDF with the same set of samples. For the NDF-A procedure, 4 mL of ANKOM heat stable α—amylase (ANKOM Technology FAA) with activity level of 340-374 MWU mL^{-1} was added to each of the first three rinses. All other steps and solutions were the same as used for the filter bag NDF method (NDF-FB). The NDF and ADF concentrations were calculated as follows:

$$\text{NDF or ADF (g kg}^{-1}) = \text{(post-digestion dry wt / pre-digestion dry wt)} \times 1000.$$

The conventional (C) IVDMD procedure used in this study was the direct acidification method with the Kansas State buffer described by Marten and Barnes (1980). Filter paper filtration was used for the conventional IVDMD analysis. The same rumen fluid was used for conventional and filter bag procedures. Rumen fluid was a 50-50 mix of fluid from a steer on a high quality alfalfa (*Medicago sativa* L.) diet and from a steer on a low quality diet that consisted primarily of ground corn cobs. Procedures for processing and preparing the rumen fluid inoculum and the rumen fluid buffer solution are fully described by Marten and Barnes (1980).

The ANKOM Rumen Fermenter (Model No: Daisy II) consists of a constant temperature cabinet that contains four glass fermentation vessels that are placed on rotation racks in the cabinet and was used for the fiber bag IVDMD analyses with ANKOM F57 filter bags. The procedures that we used for IVDMD analysis with the ANKOM system were modifications that we developed in our laboratory after several trial runs and after consultation with Dr. H.J. Jung (personal communications, 1995). Our modified procedure was as follows. Filter bags containing the ground samples were placed in glass vessels of the Rumen Fermenter that have enclosed plastic separation panels with holes. We placed the 23 samples listed in Table 1, two standard samples, and one blank (empty filter bag) into each vessel. The buffer solution (1600 mL) and rumen inoculum fluid (400 mL) was added to each vessel, the vessels were purged with CO_2, lids with gas relief valves were placed on the vessels, and the vessels were placed in the incubator for 48 h where the temperature was maintained at 39°C. The jars were mounted on slow turning rollers inside the

Table 2. Analysis of variance comparing filter bag vs. conventional analyses of cool-season forage, perennial and annual warm-season forage for NDF, ADF, and IVDMD.

		Mean squares for traits								
		All samples					Bromegrass			
Source	df	NDF-A	NDF	ADF	IVDMD	df	NDF-A	NDF	ADF	IVDMD
		g kg^{-1}					g kg^{-1}			
Rep	2	2 602	2 658	26 728	3 912*	2	781	1 171*	5 738	941
Method†	1	177 482**	7 612	18 158	49 371*	1	72 579**	677	8 091	23 577**
Entry	22	25 650**	20 234**	11 010**	35 686**	7	2 879**	1 867**	2 874**	17 292**
Method × entry	22	551*	862**	303	1 532	7	321	756	362	1 232
Rep × method	2	381	4 090**	69 461**	593	2	959*	1 954**	14 757**	209
Rep × entry	44	180	205	249	1 058	14	293	297	270	581
Error	44	288	237	399	1 102	14	216	287	494	869
CV%		2.6	2.3	5.1	5.7		2.4	2.6	6.0	4.9
		Switchgrass					Forage sorghum			
	df	NDF-A	NDF	ADF	IVDMD	df	NDF-A	NDF	ADF	IVDMD
		g kg^{-1}					g kg^{-1}			
Rep	2	832	801	11 875	6 941	2	1 189	897*	10 914	1 095
Method	1	65 081**	16 549*	4 931	11 092	1	42 554**	2	5 336	15 546
Entry	6	9 699**	10 668**	8 126**	28 921**	7	45 047**	35 797**	19 998**	33 198**
Method × entry	6	88	231	221	3 346	7	944**	382	371	595
Rep × method	2	398	401	21 508**	1 378	2	385	2 336**	35 877**	1 313
Rep × entry	12	128	117	178	1 783	14	134	218	152	492
Error	12	388	220	218	2 167	14	163	183	195	408
CV%		2.8	2.1	3.6	8.9		2.1	2.1	3.5	3.2

*,** Indicate statistical significance at the 0.05 and 0.01 levels of probability, respectively.
† Error mean square for method and entry were method × rep and entry × rep, respectively.

fermentation cabinet which results in vessel rotation and filter bag agitation.

At the completion of the 48-h incubation period, the rumen fluid was drained from the vessels and the filter bags were gently squeezed against the sides of the jar to remove the gas trapped in the inflated bags. The bags were rinsed in the jars with three changes of warm tap water. Following the last rinse, 2000 mL of acid pepsin solution (Marten and Barnes, 1980) was added to the jars and they were returned to the incubator for another 48 h. At the end of the acid pepsin digestion, the filter bags and enclosed samples were rinsed four times with tap water, dried for 48 h in a 60°C oven, and weighed. The concentration of IVDMD was calculated as follows:

$$\text{IVDMD (g kg}^{-1}) = 1 - (\text{post-digestion dry wt} / \text{pre-digestion dry wt}) \times 1000.$$

The experimental design was a randomized complete block with three replicates which were separate runs. Methods and entries were fixed effects. Analysis of variance was conducted over all samples and for each sample subset, i.e., bromegrass, switchgrass, and forage sorghum.

Results and Discussion

There were significant differences among forage samples for NDF, NDF-A, ADF, and IVDMD for the complete set of forage samples and for each of the three subsets of forage samples (Table 2). However, there were no significant differences between conventional vs. filter bag methods for ADF and NDF except for switchgrass NDF (Table 2). Averaged over all samples, the filter bag NDF and ADF values were 15 and 25 g kg^{-1} lower, respectively, than the conventional NDF and ADF values. The small differences between filter bag and conventional NDF and ADF values are consistent with the results of Komarek (1993) and Komarek et al. (1994), who reported that ADF and NDF means for the filter bag and conventional methods differed by less than 2%. However, NDF-A filter bag values were significantly lower (71 g kg^{-1}) than conventional NDF values.

There were no significant differences between methods for switchgrass and forage sorghum IVDMD but there were significant differences between IVDMD methods for the bromegrass samples and the combined set of samples (Table 2). The filter bag IVDMD values were 39 and 47 g kg^{-1} higher than the conventional IVDMD values for the combined set of samples and bromegrass, respectively (Table 1).

There were no significant entry × method effects for NDF, ADF, and IVDMD except for all-sample NDF and in this instance the mean squares for the interaction effect was 23 times smaller than the entry effect mean squares. These results indicate that the NDF, ADF, and IVDMD values produced by the two methods were consistent over entries. The mean standard deviations for the filter bag procedures was similar to or lower than the mean standard deviation for conventional analysis for NDF, ADF, and IVDMD (Table 1) indicating consistency between runs with the filter bag methods.

Spearman rank correlations were used to determine if the procedures ranked the samples in a similar order (Table 3). The Spearman correlation coefficients were high and statistically significant except for bromegrass NDF. The bromegrass C2 samples had similar NDF values to the C1 samples (Table 1) so small method differences in NDF resulted in changes in relative rank. If the bromegrass samples are subdivided into C1 and C2 subsets, the relative NDF rankings for the two methods are more consistent. These results indicate that both conventional and filter bag procedures ranked samples in the same relative order for ADF, IVDMD, and NDF for the three classes of forage analyzed in this study. Pearson correlations produced similar results. The NDF-A filter bag method ranked samples in the same order as the conventional NDF method although mean

Table 3. Pearson and Spearman correlation coefficients (*r*) of filter bag and conventional NDF, ADF, and IVDMD analyses for forage samples.

Method	*n*	Pearson	Spearman
		r	
All samples			
NDF-A	23	0.96**	0.94**
NDF	23	0.93**	0.89**
ADF	23	0.94**	0.93**
IVDMD	22	0.92**	0.91**
Bromegrass			
NDF-A	8	0.80*	0.74*
NDF	8	0.43	0.64
ADF	8	0.73*	0.83**
IVDMD	8	0.91**	0.90**
Switchgrass			
NDF-A	7	0.98**	0.96**
NDF	7	0.96**	0.89**
ADF	7	0.96**	0.82*
IVDMD	7	0.83**	0.86**
Forage sorghum			
NDF-A	8	0.97**	0.88**
NDF	8	0.98**	0.93**
ADF	8	0.96**	0.98**
IVDMD	7	0.96**	0.95**

*,** Indicate statistical significance at the 0.05 and 0.01 levels of probability, respectively.

values differed significantly. These results indicate that the NDF-A method should not be used with forages that do not contain a grain component.

According to ANKOM Technology, the F57 filter bags retain 95% of particles 30×10^{-3} mm or larger when subjected to solution flow consistent with the ANKOM Fiber Analyzers (Andy Komark, personal communication, 17 March, 1997). The small differences between conventional and filter bag NDF, ADF, and IVDMD values could be due either to small particles of less than 30×10^{-3} mm in diameter escaping from the filter bags or because of increased solubilization or digestion of samples in the filter bags because of better solution-solid contact. Regardless, the results of this study indicate that forage analysis results obtained with filter bag NDF, ADF, and IVDMD procedures are similar and consistent with the results obtained with conventional procedures.

The filter bag system has distinct advantages in comparison to the conventional systems of analysis. The filter bag system for NDF, ADF, and IVDMD analysis is easier to use since all analysis are done on a bulk basis. The filter bag equipment also takes less laboratory space. Use of this system also improves laboratory safety by reducing the need to handle hot chemicals.

Acknowledgments

The authors thank Dr. H.G. Jung, USDA-ARS Dairy Scientist, at St. Paul, MN, for his helpful discussions on the filter bag system for forage analysis.

References

Anonymous. 1995a. Acid detergent and neutral detergent fiber using ANKOM's fiber analyzer F200. Ankom Technology Corporation, Fairport, NY.

Anonymous. 1995b. In vitro true digestibility using ANKOM's DAISY^{II}. Ankom Technology Corporation, Fairport, NY.

Goering, H.K., and P.J. Van Soest 1970. Forage fiber analysis. USDA Agric. Handbook No. 379. U.S. Print. Office, Washington, DC.

Komarek, A.R. 1993. A filter bag procedure for improved efficiency of fiber analysis. J. Dairy Sci. Vol. 76, Supplement 1. p. 250.

Komarek, A.R., J.B. Robertson, and P.J. Van Soest. 1994. A comparison of methods for determining ADF using the filter bag technique versus conventional filtration. J. Dairy Sci. Vol. 77, Supplement 1. p. 114.

Marten, G.C., and R.F Barnes. 1980. Prediction of energy digestibility of forages with in vitro rumen fermentation and fungal enzyme systems. p. 61–71. *In* W.G. Pigden et al. (ed.) Standardization of analytical methodology for feeds. Publ, IDRC-134e. Int. Res. Dev. Ctr., Ottawa, ON.

Traxler, M.J., J.B. Robertson, P.J. Van Soest, D.G. Fox, A.N. Pell, and A.R. Komarek. 1995. A comparison of methods for determining IVDMD at three time periods using the Filter Bag Technique versus conventional methods. J. Dairy Sci. Vol. 78, Supplement 1. p. 274.

Vogel, K.P., and D.A. Sleper. 1994. Alteration of plants via genetics and plant breeding. p. 891–921. *In* G.C. Fahey, Jr. (ed.) Forage quality, evaluation, and utilization. ASA, CSSA, and SSSA. Madison, WI.

Van Soest, P.J., and J.B. Robertson. 1980. Systems of analysis for evaluation of fibrous feeds. p. 49–60. *In* W.G. Pigden et al. (ed.) Standardization of analytical methodology for feeds. Publ. IDRC-134e. Int. Res. Dev. Ctr., Ottawa, ON.